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Drug-induced toxicity on mitochondria and lipid metabolism: Mechanistic diversity and deleterious consequences for the liver

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Numerous investigations have shown that mitochondrial dysfunction is a major mechanism of drug-induced liver injury, which involves the parent drug or a reactive metabolite generated through cytochromes P450. Depending of their nature and their severity, the mitochondrial alterations are able to induce mild to fulminant hepatic cytolysis and steatosis (lipid accumulation), which can have different clinical and pathological features. Microvesicular steatosis, a potentially severe liver lesion usually associated with liver failure and profound hypoglycemia, is due to a major inhibition of mitochondrial fatty acid oxidation (FAO). Macrovesicular steatosis, a relatively benign liver lesion in the short term, can be induced not only by a moderate reduction of mitochondrial FAO but also by an increased hepatic *de novo* lipid synthesis and a decreased secretion of VLDL-associated triglycerides. Moreover, recent investigations suggest that some drugs could favor lipid deposition in the liver through primary alterations of white adipose tissue (WAT) homeostasis. If the treatment is not interrupted, steatosis can evolve toward steatohepatitis, which is characterized not only by lipid accumulation but also by necroinflammation and fibrosis. Although the mechanisms involved in this aggravation are not fully characterized, it appears that overproduction of reactive oxygen species by the damaged mitochondria could play a salient role. Numerous factors could favor drug-induced mitochondrial and metabolic toxicity, such as the structure of the parent molecule, genetic

predispositions (in particular those involving mitochondrial enzymes), alcohol intoxication, hepatitis virus C infection, and obesity. In obese and diabetic patients, some drugs may induce acute liver injury more frequently while others may worsen the pre-existent steatosis (or steatohepatitis).

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Introduction

More than a 1000 drugs of the modern pharmacopoeia can induce liver injury with different clinical presentations [1,2]. In the most severe cases, drug-induced liver injury (DILI) can require liver transplantation or lead to the death of the patient [3]. In addition, DILI can lead to the withdrawal of drugs from the market or earlier during clinical trials, thus causing huge financial losses. A recent retrospective study indicates that the risk of DILI is enhanced when the administered daily dosage is higher than 50 mg or when the drug undergoes significant liver metabolism [4].

The mechanisms of DILI are not always known, but when they are investigated mitochondrial dysfunction is often present [5–7]. Importantly, drug-induced mitochondrial dysfunction can be due to the drug itself and/or to reactive metabolites generated through cytochrome P450-mediated metabolism [5,6,8]. Mitochondrial dysfunction is a generic term, which includes alteration of different metabolic pathways and damage to mitochondrial components. In addition, these mitochondrial disturbances can have a variety of deleterious consequences, such as oxidative stress, energy shortage, accumulation of triglycerides (steatosis), and cell death. Regarding steatosis, recent investigations suggest that besides mitochondrial dysfunction several other mechanisms could be involved. Before discussing the main mechanisms involved in drug-induced mitochondrial dysfunction and lipid dysmetabolism, we shall recall some important features pertaining to the central role of mitochondria in cell death and energy homeostasis. We will also bring to mind some aspects of lipid metabolism not directly related to mitochondria and the most relevant effects of the adipose hormones adiponectin and leptin on liver function. Finally, this review will also evoke the main factors that could predispose some patients to DILI, in particular when hepatotoxicity is due to mitochondrial dysfunction or due to impaired lipid homeostasis.

Keywords: Hepatotoxicity; Drugs; Mitochondria; Steatosis; Lipids; Cell death; Obesity; Oxidative stress.

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Abbreviations: ACC, acetyl-CoA carboxylase; APAP, acetaminophen; AZT, zidovudine; CAR, constitutive androstane receptor; ChREBP, carbohydrate responsive element-binding protein; CoA, coenzyme A; CPT, carnitine palmitoyltransferase; CYP, cytochrome P450; ddl, didanosine; d4T, stavudine; DILI, drug-induced liver injury; FAO, fatty acid oxidation; GSH, reduced glutathione; GST, glutathione S-transferase; JNK, c-Jun-N-terminal kinase; LCFA, long-chain fatty acid; MPTP, mitochondrial permeability transition pore; MTP, microsomal triglyceride transfer protein; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; NAFLD, nonalcoholic fatty liver disease; NAPQI, N-acetyl-p-benzoquinone imine; NASH, nonalcoholic steatohepatitis; NRTI, nucleoside reverse transcriptase inhibitor; OXPHOS, oxidative phosphorylation; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element-binding protein-1c; TNF α , tumor necrosis factor- α ; TCA, tricarboxylic acid cycle; TZD, thiazolidinedione; VPA, valproic acid; VLDL, very-low density lipoprotein; WAT, white adipose tissue.



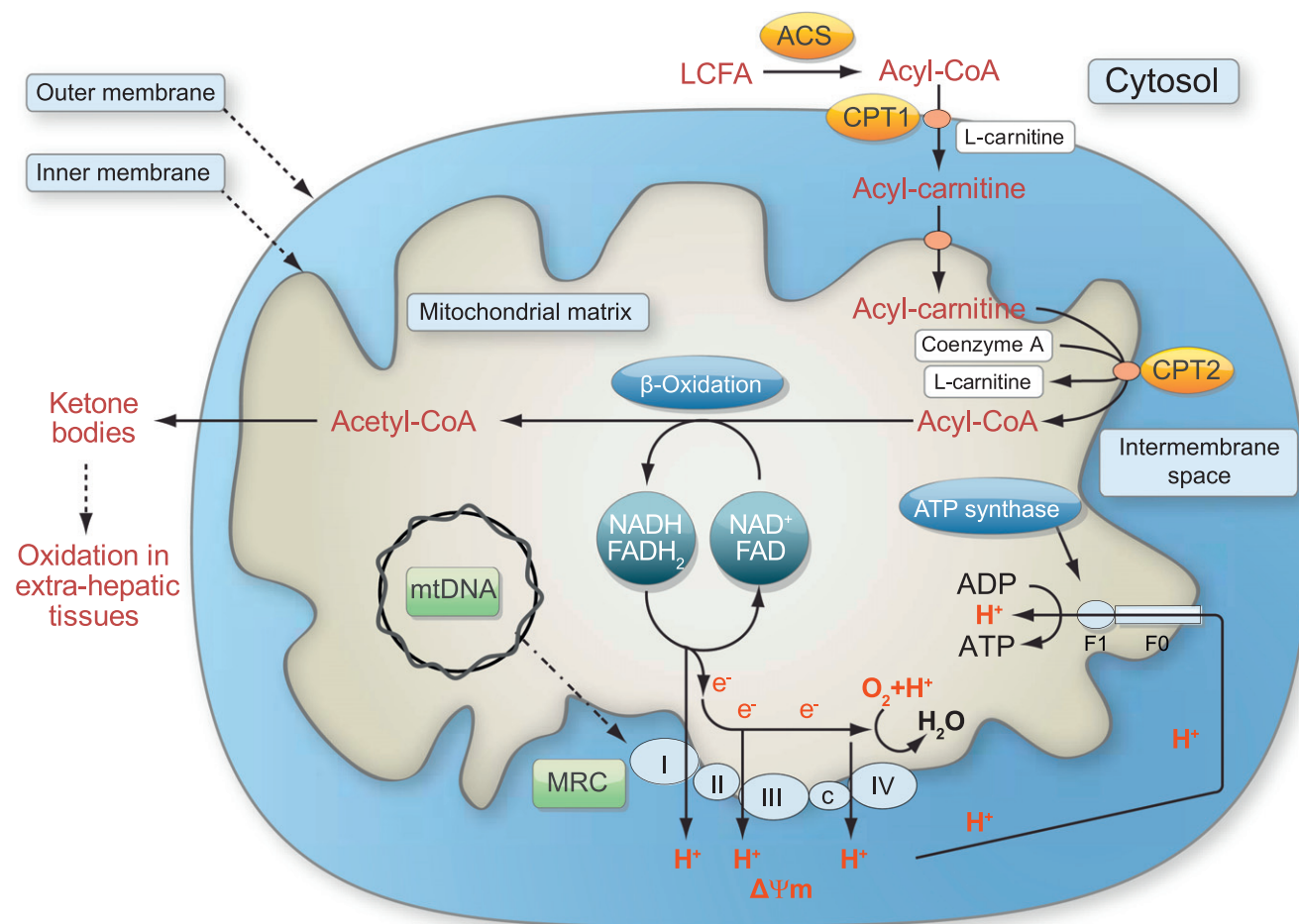


Fig. 1. Schematic representation of mitochondrial fatty acid β -oxidation and oxidative phosphorylation in liver mitochondria. In contrast to short-chain and medium-chain fatty acids (not shown), the entry of long-chain (C_{14} – C_{18}) fatty acid (LCFA) within mitochondria requires a specific shuttle system involving four steps. (A) LCFAs are activated into LCFA-coenzyme A (acyl-CoA) thioesters by long-chain acyl-CoA synthetases (ACS) located in the outer mitochondrial membrane. (B) The long-chain acyl-CoA is converted into an acyl-carnitine derivative by carnitine palmitoyltransferase-1 (CPT 1) in the outer mitochondrial membrane. (C) This acyl-carnitine derivative is then translocated across the inner mitochondrial membrane into the mitochondrial matrix by carnitine-acylcarnitine translocase. (D) Finally, carnitine palmitoyltransferase-2 (CPT 2), located on the matrix side of the inner mitochondrial membrane, transfers the acyl moiety from carnitine back to coenzyme A. LCFA-CoA thioesters are then oxidized into acetyl-CoA moieties via the β -oxidation process. Acetyl-CoA moieties directly generate ketone bodies (mainly acetoacetate and β -hydroxybutyrate) which are liberated into the plasma to be used by extra-hepatic tissues for energy production. Mitochondrial fatty acid oxidation (FAO) generates NADH and FADH₂, which transfer their electrons (e^-) to the mitochondrial respiratory chain (MRC), thus regenerating NAD⁺ and FAD used for other β -oxidation cycles. Within the MRC, electrons are sequentially transferred to different polypeptide complexes (numbered from I to IV) embedded within the inner membrane. The final transfer of the electrons to oxygen takes place at the level of complex IV which oxidizes cytochrome c (c). The flow of electrons within the MRC is coupled with the extrusion of protons (H^+) from the mitochondrial matrix to the intermembrane space, which creates the mitochondrial transmembrane potential, $\Delta\psi_m$. When energy is needed (i.e. when ATP levels are low), these protons re-enter the matrix through the F₀ portion of the ATP synthase (also referred to as complex V), thus liberating energy that is used to phosphorylate ADP into ATP. The whole metabolic process which couples substrate oxidation to ATP synthesis is referred to as oxidative phosphorylation (OXPHOS). It is noteworthy that OXPHOS requires the mitochondrial DNA (mtDNA) since it encodes 13 MRC polypeptides, which are embedded within complexes I, III, IV, and V.

Mitochondrial structure and functions

Mitochondrial membrane permeabilization and cell death

Mitochondria are organelles with two membranes surrounding a space (matrix) containing various enzymes and the mitochondrial genome (mtDNA) (Fig. 1). The inner membrane, which also harbors many enzymes, behaves as a barrier that is poorly permeable to various molecules [9]. Thus, this membrane contains transporters allowing the entry of endogenous compounds (ADP, fatty acids, glutathione, pyruvic acid) and possibly xenobiotics as well.

In some pathophysiological circumstances, the mitochondrial membranes can lose their structural and functional integrity, in

particular after the opening of the mitochondrial permeability transition pores (MPTP) [10]. These pores involve at least 4 candidate proteins, namely the peripheral benzodiazepine receptor (PBR), the voltage-dependent anion channel (VDAC), the adenine nucleotide translocase (ANT), and cyclophilin D [10]. The later protein (a modulator of the pore rather than a MPTP component *per se* [11]) is able to bind the immunosuppressive drug cyclosporin A that therefore reduces the opening probability of the MPTP. In contrast, several drugs and toxic compounds, but also high levels of some endogenous derivatives (e.g. calcium, fatty acids, and bile salts) can induce MPTP opening. As the latter event strongly alters mitochondrial function and structure, it can endanger cell life. However, the exact pathway whereby the cell

will die (namely apoptosis or necrosis) depends on the number of mitochondria harboring opened MPTP [6,7,12].

Indeed, MPTP opening can profoundly disturb ATP synthesis, through the loss of inner mitochondrial membrane integrity. If numerous mitochondria present opened MPTP, ATP stores will slump rapidly and necrosis will occur through a sudden rise in intracellular calcium levels because ATP is mandatory for the activity of the plasma membrane calcium ATPase (PMCA), an enzyme responsible for calcium extrusion out of the cell. In contrast, if MPTP opening takes place only in some mitochondria, ATP levels will be maintained thanks to undamaged organelles. However, the rare mitochondria involved in MPTP opening will swell allowing the release of different pro-apoptotic proteins including the apoptosis inducing factor (AIF), several caspases, and cytochrome *c* [13]. This key protein of the respiratory chain (Fig. 1), when released in the cytoplasm, can bind to the Apaf-1 protein and ATP thus initiating the apoptotic pathway through the activation of caspases 9 and 3. Consequently, MPTP opening in a few mitochondria can also have deleterious consequences [12,14].

Several important points must be discussed regarding mitochondrial membrane permeabilization. Firstly, MPTP opening initially permeabilizes the mitochondrial inner membrane without alteration of the outer membrane. However, MPTP opening causes an equilibration of solutes with molecular masses up to 1500 Da and the massive entry of water into the matrix, which causes unfolding of the inner membrane and mitochondrial swelling. The latter event thus induces outer membrane rupture and the release of several mitochondrial proteins located in the intermembrane space (e.g. cytochrome *c* and AIF), which trigger apoptosis [10,13,15]. Secondly, mitochondrial membrane permeabilization can induce the release of cytochrome *c* and other cytotoxic proteins without any rupture of the mitochondrial outer membrane [13,16]. This scenario requires the formation of pores within this membrane thanks to the association of two pro-apoptotic proteins belonging to the Bcl-2 family, namely Bak (already located in the outer membrane) and Bax (which is recruited from the cytosol) [10,13]. Importantly, mitochondrial outer membrane permeabilization through the formation of Bax/Bak pores is not sensitive to cyclosporin A [17,18]. Thus, whatever the mechanism involved in membrane permeabilization, this event can strongly alter mitochondrial function and structure, and thus lead to cell death. Finally, it is noteworthy that the MPTP structure seems to be different from one tissue to another. This may explain why some organs could be more or less vulnerable to certain permeability transition inducers [19,20].

Liver mitochondria and energy homeostasis

In most mammalian cells, mitochondria provide the most part of the energy necessary for cell homeostasis, especially during fasting periods [5,21,22]. Mitochondrial ATP synthesis is possible thanks to the oxidative degradation of endogenous substrates, such as pyruvate (generated from glycolysis), fatty acids, and amino acids. Pyruvate oxidation takes place in the tricarboxylic acid cycle (TCA, also called Krebs cycle), whereas fatty acid degradation within mitochondria is mediated by β -oxidation (Fig. 1).

In order to undergo the β -oxidation pathway fatty acids must cross the mitochondrial membranes. Whereas short-chain and medium-chain fatty acids freely enter the mitochondria, long-chain fatty acids (LCFAs) can cross the mitochondrial membranes

only by means of a multienzymatic system requiring coenzyme A and L-carnitine as cofactors (Fig. 1). In this system, carnitine palmitoyltransferase 1 (CPT1) catalyses the rate limiting step of LCFA oxidation as this enzyme can be strongly inhibited by malonyl-CoA, an endogenous derivative synthesized during *de novo* lipogenesis [23,24].

Inside the mitochondria, short-chain and medium-chain fatty acids are activated in acyl-CoA molecules by specific acyl-CoA synthetases, whereas long-chain fatty acyl-carnitine intermediates are transformed back to their corresponding acyl-CoA thioesters thanks to CPT2 (Fig. 1). Whatever the length of their carbon chain, acyl-CoA derivatives are then cut down sequentially thanks to the β -oxidation process that generates acetyl-CoA moieties and shorter fatty acids that enter new β -oxidation cycles (Fig. 1). These acetyl-CoA moieties are immediately used for the synthesis of ketone bodies (mainly acetoacetate and β -hydroxybutyrate) released in the blood and oxidized in extra-hepatic tissues, such as kidney, muscle, and brain (Fig. 1). Because mitochondrial β -oxidation and ketogenesis play a fundamental role in energy homeostasis [5,25], a severe deficiency in fatty acid oxidation (FAO) can lead to multiple organ failure and death of the patient [5,6,26].

FAO deficiency can be associated with reduced plasma ketone bodies, accumulation of acyl-carnitine derivatives and dicarboxylic acids in plasma (or urine), and severe hypoglycemia [5,6,26]. Low blood glucose could be due to reduced hepatic gluconeogenesis and increased extra-hepatic utilization [5,27]. Although hypoketonemia is usually observed in genetic disorders of mitochondrial FAO, hyperketonemia can be observed during drug-induced alteration of mitochondrial β -oxidation [5,6]. A probable mechanism is the occurrence of drug-induced impairment of the TCA cycle in extra-hepatic tissues consuming high amounts of ketone bodies [5,28].

Oxidative degradation of pyruvate and fatty acids produces acetyl-CoA molecules and also reduced cofactors [5,6,9]. Indeed, several dehydrogenases involved in the TCA cycle and β -oxidation are using NAD⁺ and FAD to generate NADH and FADH₂, which give their electrons and protons to the mitochondrial respiratory chain (MRC) (Fig. 1). Electrons are sequentially transferred to different multi-protein complexes of the MRC and finally to cytochrome *c* oxidase (complex IV), which safely reduces oxygen into water in the presence of protons (Fig. 1). Importantly, electron transfer within MRC is associated with the ejection of protons from the matrix to the intermembrane space of the mitochondria, thus generating a large membrane potential $\Delta\psi_m$ [9,29]. When cells need energy, protons are reentering the matrix thanks to the F₀ portion of the ATP synthase (complex V) thus releasing part of the potential energy of $\Delta\psi_m$. This energy is then used by the F₁ portion of the ATP synthase for the phosphorylation of ADP into ATP (Fig. 1). Some drugs able to abolish ADP phosphorylation (and thus ATP synthesis) without inhibiting substrate oxidation are referred to as oxidative phosphorylation (OXPHOS) uncouplers [5,6,30].

Mitochondrial production of reactive oxygen species

A major feature of the mitochondria is the production of reactive oxygen species (ROS) through the activity of the MRC [22,31]. Indeed, a small fraction of electrons entering the MRC can prematurely escape from complexes I and III and directly react with

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oxygen to generate the superoxide anion radical. This radical is then dismutated by the mitochondrial manganese superoxide dismutase (MnSOD) into hydrogen peroxide (H_2O_2), which is detoxified into water by the mitochondrial glutathione peroxidase (GPx) that uses reduced glutathione (GSH) as a cofactor. Hence, in the normal (non-diseased) state, most of the ROS generated by the MRC are detoxified by the mitochondrial antioxidant defenses. The remaining (i.e. non-detoxified) ROS diffuse out of mitochondria and serve as second messengers to trigger cellular processes such as mitogenesis [22].

However, this detoxification process can be overwhelmed in different pathophysiological circumstances. This occurs in particular in case of GSH depletion within liver mitochondria, which reduces greatly their capability to detoxify H_2O_2 since they do not have catalase [32]. Depletion of mitochondrial GSH below a critical threshold thus favors H_2O_2 accumulation by impairing its detoxification. This in turn triggers mitochondrial dysfunction, MPTP opening, activation of c-Jun-N-terminal kinase (JNK), and cell death [33,34]. Chronic ethanol intoxication, fasting, and malnutrition are diseased states favoring GSH depletion, in particular within mitochondria.

Mitochondrial anti-oxidant enzymes can also be overwhelmed when MRC is chronically impaired. Indeed, a partial block in the flow of electrons greatly increases the probability of monoelectronic reduction of oxygen and superoxide anion production within the complexes I and III [35,36]. High steady state levels of ROS then damage OXPHOS proteins, cardiolipin, and mtDNA [37–39]. This oxidative damage aggravates mitochondrial dysfunction to further augment electron leakage and ROS formation, thus leading to a vicious circle [40].

The mitochondrial genome

A unique feature of mitochondria is the dual genetic origin of the OXPHOS proteins (ca. 100) [5,22]. Whereas the most part of these polypeptides are encoded by the nuclear genome and subsequently imported within the mitochondria, 13 MRC polypeptides are instead encoded by the mitochondrial genome, a small piece of circular doubled-stranded DNA located within the mitochondrial matrix (Fig. 1). In a single cell there are several hundred (or thousand) copies of mtDNA whose replication occurs continuously, even in cells that do not divide [41,42]. Permanent mtDNA replication by the DNA polymerase γ thus allows the maintenance of constant mtDNA levels in cells despite continuous removal of the most dysfunctional and/or damaged mitochondria [43].

Most cells (including hepatocytes) have a surplus of mtDNA copies, and can, therefore, tolerate a substantial depletion of mtDNA. Classically, it is considered that the number of normal mtDNA copies must fall below 20–40% of basal levels to induce mitochondrial dysfunction and severe adverse events [41,44,45]. The few mtDNA copies remaining within each mitochondrion are not able to provide enough MRC polypeptides, thus leading to OXPHOS impairment and secondary inhibition of mitochondrial FAO and TCA cycle. Another key feature of mtDNA is its high sensitivity to ROS-induced oxidative damage and mutations due to its proximity to the inner membrane (a major source of ROS), the absence of protective histone, and an incomplete repertoire of mitochondrial DNA repair enzymes [37,41,46,47].

Lipid and carbohydrate metabolism in extramitochondrial compartments

Besides mitochondria, other organelles (or extra-mitochondrial enzyme systems) can be involved in FAO. For instance, peroxisomes degrade long-chain and very long-chain fatty acids but not medium-chain and short-chain fatty acids. The first step of peroxisomal FAO continuously generates H_2O_2 through acyl-CoA oxidase (ACO) activity [48,49], and thus oxidative stress can occur during fatty acid overload and/or peroxisomal proliferation due to an imbalance between intraperoxisomal H_2O_2 production and its removal by catalase [50]. Several cytochromes P450 (CYPs) such as CYP4A and CYP2E1 also oxidize fatty acids although the CYP-mediated oxidation involves only the terminal ω (or the $\omega-1$) carbon of the aliphatic chain [51,52]. Interestingly, ω -hydroxylated fatty acids are further converted into dicarboxylic acids that can induce mitochondrial dysfunction [5,53]. Although most of the CYPs are found within the endoplasmic reticulum, some of them such as CYP2E1 can have a mitochondrial localization [54–56].

Mitochondrial, peroxisomal, and microsomal FAO is strongly regulated by peroxisome proliferator-activated receptor α (PPAR α), a nuclear receptor and transcription factor, which can be stimulated by endogenous fatty acids or synthetic drugs (fibrates) [57]. PPAR α stimulation increases the expression of the mitochondrial enzymes CPT1, medium-chain acyl-CoA dehydrogenase (MCAD) and HMG-CoA synthase (involved in ketone body synthesis), the peroxisomal ACO, and the microsomal CYP4A [58,59]. Besides PPAR α , other transcription factors regulating hepatic FAO include forkhead box A2 (FoxA2) and cAMP-response element-binding protein (CREB) that are activated during fasting periods by low insulinemia and high glucagonemia, respectively [60].

On the contrary, the metabolic and hormonal context after a meal favors lipid synthesis with a concomitant reduction of the FAO pathway. Indeed, high plasma levels of insulin and glucose, respectively, activate the sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate responsive element-binding protein (ChREBP) that both increase the hepatic expression of key enzymes involved in glycolysis (e.g. glucokinase and L-pyruvate kinase) and *de novo* lipogenesis (e.g. acetyl-CoA carboxylase and fatty acid synthase). Lipogenesis is associated with the accumulation of the CPT1 inhibitor malonyl-CoA, thus reducing the flux of mitochondrial LCFA oxidation [23,24].

It is worthy to mention herein that hepatic SREBP-1c and ChREBP can be abnormally activated in obese and diabetic individuals thus favoring fatty liver. Another mechanism that could contribute to fatty liver in these patients is the permanent and unrepressed triglycerides lipolysis taking place in the expanded adipose tissue (due to insulin resistance), which leads to a massive influx of free fatty acids in the hepatocytes [60]. Besides SREBP-1c and ChREBP, other transcription factors could play a significant role in *de novo* lipogenesis (at least in some metabolic contexts) such as PPAR γ and pregnane X receptor (PXR). Both transcription factors are nuclear receptors that can be activated by different endogenous and exogenous ligands [61,62].

Once synthesized, fatty acids combine with glycerol to generate triglycerides. These lipids are subsequently incorporated into VLDL particles, which are normally secreted into the plasma unless this route of lipid secretion is impaired. VLDL synthesis requires not only triglycerides but also apolipoproteins B and CIII.

Furthermore, VLDL assembly within the endoplasmic reticulum requires the microsomal triglyceride transfer protein (MTP) whose expression is reduced by insulin [63]. In the plasma, VLDL particles are hydrolyzed by lipoprotein lipase (LPL), thus allowing the release of free fatty acids that will be either oxidized in different extra-hepatic tissues (e.g. heart, skeletal muscles) or re-esterified into triglycerides in the adipose tissue. LPL is usually not expressed in the adult liver except in some pathophysiological situations such as obesity [64].

Impact of leptin and adiponectin on lipid and carbohydrate metabolism

Besides insulin and glucagon, hormones secreted by the adipose tissue (referred to as adipokines) can also play a salient role in lipid homeostasis. Among these adipokines, leptin, and adiponectin present an “anti-steatotic” action by decreasing *de novo* lipogenesis and activating mitochondrial FAO, in particular by reducing the intracellular levels of malonyl-CoA [65,66]. Indeed, leptin and adiponectin can induce the phosphorylation of the lipogenic enzyme acetyl-CoA carboxylase (ACC), thus leading to its inactivation and the subsequent reduction of malonyl-CoA synthesis [66,67]. Both adipokines also control carbohydrate homeostasis in several tissues including the liver [67,68].

Leptin also strongly regulates food intake. Consequently, low leptinaemia can induce obesity and associated metabolic disorders, such as dyslipidemia, type 2 diabetes, and fatty liver [66,69,70]. However, total leptin deficiency is particularly rare in humans. In contrast, common obesity is associated with high leptinemia (a consequence of leptin resistance) and low adiponectinemia, which plays a major role in the pathophysiology of type 2 diabetes and fatty liver [71,72]. Finally, while leptin favors inflammation, fibrogenesis, and angiogenesis, adiponectin prevents these different events [71].

Drug-induced mitochondrial dysfunction and liver injury

Drug-induced adverse events and mitochondrial toxicity

The view that drugs could disturb mitochondrial function emerged several decades ago when clinical studies reported in some medicated individuals the occurrence of symptoms usually observed in patients presenting a mitochondrial disease of genetic origin or a Reye's syndrome (whose physiopathology involves severe mitochondrial dysfunction) [5]. For instance, several studies reported in the late 70's and early 80's the occurrence of a Reye-like syndrome in epileptic patients treated with valproic acid (VPA) [73,74]. Likewise, myopathy, lactic acidosis,

Table 1. Hepatotoxic drugs and their corresponding deleterious effects on mitochondrial function and genome. Note that the absence of cross indicates that the toxic effect has not been reported to date for the corresponding drug and that for different compounds listed below some of the mitochondrial effects have been observed only *in vitro*.

Drug	MPTP ^a opening	Direct inhibition of mitochondrial FAO ^b	OXPHOS uncoupling	Direct inhibition of the MRC ^c	mtDNA depletion or damage
Acetaminophen (APAP) ^d	+			+	+
Alpidem	+		+	+	
Amineptine		+			
Amiodarone	+	+	+	+	
Buprenorphine		+	+	+	
Diclofenac	+	+	+		
Didanosine (ddI)					+
Disulfiram	+			+	
Fialuridine (FIAU)					+
Ibuprofen		+	+		
Nilutamide				+	
Nimesulide	+		+		
Panadiplon		+			
Perhexiline		+	+	+	
Pirprofen		+			
Salicylic acid	+	+	+		
Stavudine (d4T)					+
Tacrine			+		+
Tamoxifen		+	+	+	+
Tetracycline (and its derivatives)				+	
Troglitazone	+			+	+
Valproic acid (VPA)	+	+			
Zidovudine (AZT)					+

^aAbbreviations: FAO, fatty acid oxidation; MPTP, mitochondrial permeability transition pores; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation.

^bInhibition of mitochondrial FAO through impairment of FAO enzyme(s) and/or depletion in L-carnitine and coenzyme A.

^cInhibition of the MRC through impairment of enzyme(s) involved in electron transfer or ADP phosphorylation.

^dMitochondrial effects of APAP via its reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI).

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and hepatic steatosis have been reported in the late 80's and early 90's in patients treated with the antiretroviral nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine (AZT), zalcitabine (ddC), didanosine (ddI) and stavudine (d4T) [5,75–77]. Since then, the list of drugs inducing adverse events due to mitochondrial dysfunction has not ceased to grow year after year.

Regarding drug-induced liver diseases, different mechanisms of mitochondrial dysfunction have been described thus far, including membrane permeabilization, OXPHOS impairment, FAO inhibition, and mtDNA depletion (Table 1) [5–7]. Importantly, DILI due to mitochondrial toxicity has led to the interruption of clinical trials, or drug withdrawal after marketing, in particular when the benefit/risk ratio was deemed to be too low for the patient's healthiness (Table 2). Moreover, some marketed drugs have received Black Box warnings from drug agencies due to mitochondrial dysfunction and related hepatotoxicity (Table 3) [6,78].

Drug-induced mitochondrial alterations and cytolytic hepatitis

Cytolytic hepatitis encompasses a wide spectrum of liver injury of different severity since the destruction of hepatocytes (i.e. cytolysis) can involve a variable amount of the hepatic mass. Consequently, the mildest forms are characterized by an isolated increase in plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), whereas in the most severe cases fulminant hepatitis can occur thus requiring liver transplantation [3]. As already mentioned, hepatocyte cytolysis occurring *in vivo* can be the consequence of necrosis or apoptosis. While necrosis leads to the destruction of the plasma membrane and the release in the extracellular milieu of different cell components such as transaminases and lactate dehydrogenase (LDH), apoptosis is generally associated with a discreet removal of the dying cells by neighboring macrophages [14,79]. However, the removal of a large number of apoptotic cells can induce the recruitment of inflammatory cells and the subsequent overproduction of ROS and cytokines that promote cell necrosis [80]. Thus, apoptosis in liver can also be associated *in vivo* with secondary necrosis and elevated plasma transaminases [81,82].

Drug-induced MPTP opening

MPTP opening is one mechanism whereby drugs can induce cytolytic hepatitis (Table 1) [6,17,83–87]. Among these drugs, disulfiram can also induce mitochondrial membrane permeabilization through a MPTP-independent mechanism [17]. Studies pertain-

Table 2. Examples of drugs, the potential of which to cause mitochondrial dysfunction and DILI has led to the interruption of clinical trials, or their withdrawal after marketing.

Drug	Therapeutic class
Drug withdrawn during clinical trials	
Fialuridine	Antiviral (anti-HBV)
Panadiplon	Anxiolytic
Drug withdrawn after marketing	
Alpidem	Anxiolytic
Perhexiline	Antianginal
Pirprofen	NSAID ^a
Troglitazone	Antidiabetic (PPAR γ agonist)

^aAbbreviation: NSAID, nonsteroidal anti-inflammatory drug.

Table 3. Examples of marketed drugs able to induce hepatotoxicity due to mitochondrial dysfunction, which have received Black Box warnings from drug agencies.

Drug	Therapeutic class
Amiodarone	Antianginal, antiarrhythmic
Benzbromarone	Uricosuric
Buprenorphine	Opioid agonist/antagonist
NRTIs ^a (AZT, d4T, ddI)	Antiretroviral (anti-HIV)
Tamoxifen	Antineoplastic (breast cancer)
Tolcapone	Anti-Parkinson
Valproic acid (VPA)	Antiepileptic

^aAbbreviation: nucleoside reverse transcriptase inhibitors.

ing to drug-induced MPTP are sometimes performed in mitochondria de-energized with oligomycin and in the presence of high concentrations of calcium (e.g. from 10 to 50 μ M). Since these conditions have a profound impact on MPTP opening [10], it is difficult to extrapolate some data to the *in vivo* situation.

The precise mechanisms whereby drugs can induce MPTP opening are not known although recent investigations suggest at least three hypotheses, which are not mutually exclusive.

Firstly, drugs can interact with some MPTP components. For instance, alpidem could trigger mitochondrial membrane permeabilization and cell death through its binding to PBR which is located on the outer membrane [86].

Secondly, drug-induced oxidative stress can favor the oxidation of regulatory thiol groups located within some MPTP components [8,17,88]. This mechanism could occur with disulfiram and acetaminophen (APAP) that both induce major oxidative stress [8,17,89]. As regards APAP, it is, however, unclear whether this drug induces MPTP opening via GSH depletion, or through the direct interaction of its reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) with some (still uncharacterized) MPTP components. Indeed, NAPQI is able to bind covalently to mitochondrial proteins and this could have deleterious effect not only on MPTP but also on mitochondrial respiration and FAO [90–92].

Thirdly, drugs such as APAP and cisplatin could cause mitochondrial permeability transition through an activation of JNK or other endogenous MPTP inducers [89,93,94]. Regarding APAP, several studies suggest that JNK activation is related to ROS generation and, therefore, APAP-induced oxidative stress could promote MPTP opening through direct and indirect pathways [34,93].

Drug-induced OXPHOS impairment

Drugs can also induce cell death through a direct impairment of OXPHOS (Table 1), which reduces ATP synthesis. As already mentioned, severe ATP depletion inhibits calcium extrusion from the cell thus leading to its intracellular accumulation. This in turn activates proteases, endonucleases, and phospholipases that participate in the destruction (or the disorganization) of cell constituents including the plasma membrane and cytoskeleton, thus leading to necrosis [14,95]. In fact, drug-induced OXPHOS impairment can occur through different mechanisms.

The first mechanism is OXPHOS uncoupling without subsequent inhibition of the MRC. In this case, substrate oxidation is

maintained (since electron transfer within the MRC is not altered) although ATP synthesis is strongly hindered. Indeed, OXPHOS uncouplers are usually protonophores, namely molecules that are protonated in the mitochondrial intermembrane space thus generating cationic compounds that take advantage of the membrane potential $\Delta\psi_m$ to cross the inner membrane. Consequently, protons are entering the matrix independently of ATP synthase thus causing a drop of ATP synthesis. Drugs that induce OXPHOS uncoupling without subsequent inhibition of the MRC are for instance the nonsteroidal anti-inflammatory drug (NSAID) nimesulide and the anti-Alzheimer drug tacrine [83,96]. Other NSAIDs such as salicylic acid and ibuprofen are also OXPHOS uncouplers but their uncoupling effect is so mild that it may not induce deleterious consequences *in vivo* [5,97]. Finally, OXPHOS uncoupling can be associated with other mitochondrial effects that present a more harmful impact on cell viability. For instance, although diclofenac both uncouples OXPHOS and favors MPTP opening only the latter effect could be responsible for cell injury [98].

The second mechanism is OXPHOS uncoupling with subsequent inhibition of the MRC activity, thus leading to a secondary impairment of substrate oxidation such as FAO. Unfortunately, the precise mechanism whereby these drugs alter electron transfer within the MRC is unknown. Actually, the dual effect of some drugs on OXPHOS (i.e. uncoupling followed by inhibition) seems to be concentration-dependent and “isolated” uncoupling nevertheless can be observed for low concentrations of these drugs. Drug-induced dual effect on OXPHOS has been described with amiodarone, perhexiline, alpidem, tamoxifen, and buprenorphine [5,86,99–103]. A dual effect has also been described for salicylic acid but strong MRC inhibition induced by this drug occurs for concentrations in the millimolar range [104,105]. Finally, while drug-induced MRC blockage can participate in the inhibition of mitochondrial FAO, some drugs, such as amiodarone, perhexiline, and tamoxifen can also directly inhibit FAO enzymes such as CPT1, as discussed below [102,106,107].

A third mechanism is an inhibition of the MRC activity without any prior OXPHOS uncoupling. This situation has been described for instance with the anti-androgen drug nilutamide [108].

Drug-induced severe inhibition of mitochondrial β -oxidation and microvesicular steatosis

Some drugs can induce microvesicular steatosis (Table 4) [5,6,109–113], which is sometimes referred to as microsteatosis. Microvesicular steatosis is a potentially severe liver lesion that can be associated with liver failure, encephalopathy, and profound hypoglycemia thus leading to the death of some patients. Liver pathology shows the presence of numerous cytoplasmic lipid droplets, which can be stained with oil red O [109,114]. Hepatic cytolysis and increased plasma transaminases can also be observed to a variable degree. Amiodarone, although being able to induce “pure” microvesicular steatosis in a few patients [115,116], most often provokes macrovesicular steatosis (occasionally associated with microvesicular steatosis) and steatohepatitis. Microvesicular steatosis or mixed steatosis has seldom been reported with troglitazone in addition to other lesions, such as necroinflammation, fibrosis, and cholestasis [117–119]. Microvesicular steatosis can be also observed during ethanol intoxication, Reye’s syndrome, acute fatty liver of pregnancy, and several inborn errors of mitochondrial FAO and OXPHOS [5,109,120,121].

Table 4. Examples of drugs inducing microvesicular steatosis.

Drug	Therapeutic class
Amineptine	Antidepressant
Amiodarone	Antianginal, antiarrhythmic
2-Arylpropionic acids (pirprofen, ibuprofen)	NSAID ^a
Aspirin (and salicylic acid)	NSAID
Fialuridine (FIAU)	Antiviral (anti-HBV)
NRTIs (AZT, ddI, d4T)	Antiretroviral (ant-HIV)
Panadiplon	Anxiolytic
Tetracycline and its derivatives (high doses)	Antibiotics
Tianeptine	Antidepressant
Valproic acid (VPA)	Antiepileptic

^aAbbreviations: NRTIs, nucleoside reverse transcriptase inhibitors; NSAID, non-steroidal anti-inflammatory drug.

Whatever its etiology, microvesicular steatosis results primarily from a severe inhibition of the mitochondrial FAO (Fig. 2) [5,6,122,123]. Although other metabolic pathways could also be impaired [124], these additional mechanisms most probably play

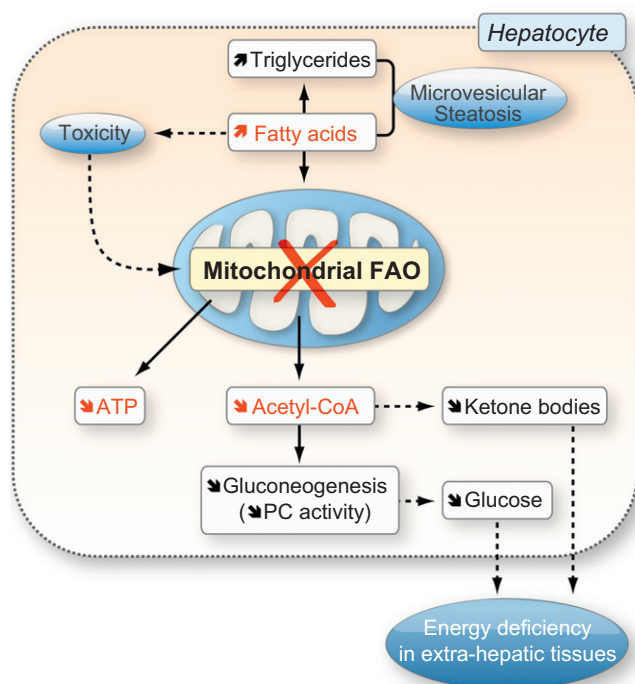


Fig. 2. Metabolic consequences of severe inhibition of mitochondrial fatty acid β -oxidation. A severe impairment of mitochondrial fatty acid oxidation (FAO) can induce accumulation of free fatty acids and triglycerides (thus explaining microvesicular steatosis), reduced ATP synthesis and lower production of ketone bodies. Inhibition of FAO also decreases gluconeogenesis through different mechanisms including lower ATP production and reduced pyruvate carboxylase (PC) activity. Low plasma levels of ketone bodies (or reduced ketone bodies utilization) and hypoglycemia are thus responsible for a profound energy deficiency in extra-hepatic tissues. The accumulation of free fatty acids (and some of their metabolites such as dicarboxylic acids) could play a major role in the pathophysiology of microvesicular steatosis. Indeed, these lipid derivatives can impair mitochondrial function through different mechanisms, thus reinforcing drug-induced inhibition of FAO.

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a secondary role in the pathophysiology and severity of microvesicular steatosis.

A primary consequence of severe inhibition of mitochondrial FAO is an accumulation of fatty acids that are either esterified into triglycerides or that remain as a free form, which can reinforce mitochondrial dysfunction (Fig. 2) [5,18,125]. Another major consequence is an impairment of energy output in the liver but also in extra-hepatic tissues attributable to lower ketone body production (or utilization). Importantly, reduced mitochondrial FAO hampers hepatic gluconeogenesis as a consequence of ATP shortage and pyruvate carboxylase inhibition, which can lead to severe hypoglycemia in some individuals (Fig. 2) [5,6]. Finally, severe impairment of mitochondrial FAO is associated with an accumulation in plasma and urines of fatty acid derivatives, such as acyl-carnitine and acyl-glycine esters and dicarboxylic acids [5,6,126].

Drug-induced severe inhibition of mitochondrial FAO can result from several mechanisms and some drugs impair this metabolic pathway by interacting with different mitochondrial enzymes [5,6]. These mechanisms can be classified into four different categories.

Firstly, drugs, such as ibuprofen, tianeptine, amiodarone, tamoxifen, and VPA can directly inhibit one or several mitochondrial FAO enzymes (Table 1) [5,102,127,128]. VPA-induced severe FAO inhibition is probably due to $\Delta 2,4$ -VPA-CoA and other reactive metabolites which irreversibly inactivate FAO enzyme(s) (Fig. 3) [129,130]. Likewise, APAP may inhibit FAO enzymes through the generation of its reactive metabolite NAPQI [91]. This

may explain why this analgesic drug induces steatosis in some individuals [1,131]. Unfortunately, the FAO enzymes inhibited by these drugs have not always been identified, although CPT1 (Fig. 1) could be a key target. Indeed, this enzyme can be inhibited by VPA (Fig. 3), amiodarone, and tamoxifen [102,107,132]. Interestingly, troglitazone is able to inhibit long-chain acyl-CoA synthase (ACS) (Fig. 1), thus impairing the mitochondrial entry of LCFAs [133].

Secondly, drugs can impair mitochondrial FAO through the generation of coenzyme A and/or L-carnitine esters, thus decreasing the levels of these major FAO cofactors (Fig. 1). This mechanism has been shown for VPA (Fig. 3), salicylic acid, and ibuprofen [5,104,134,135].

Thirdly, mitochondrial FAO can be secondarily impaired as a result of severe inhibition of the MRC [5,6]. Indeed, the MRC allows the constant regeneration of FAD and NAD⁺ required for the enzymatic reactions catalyzed, respectively, by the FAO enzymes acyl-CoA dehydrogenases and 3-hydroxyacyl-CoA dehydrogenases (Fig. 1). Inhibition of FAO secondarily to MRC impairment could occur with amiodarone (Fig. 4), perhexiline, tamoxifen, and buprenorphine [6,30,99,101,102]. Interestingly, these amphiphilic drugs can be protonated within the intermembrane space of the mitochondria thus generating cationic compounds entering the matrix thanks to the membrane potential $\Delta\psi_m$ (Fig. 4) [5,7,30,102]. Besides OXPHOS uncoupling, this allows their mitochondrial accumulation and the subsequent inhibition of both FAO and MRC enzymes. Whereas relatively low concentrations of these amphiphilic drugs can inhibit

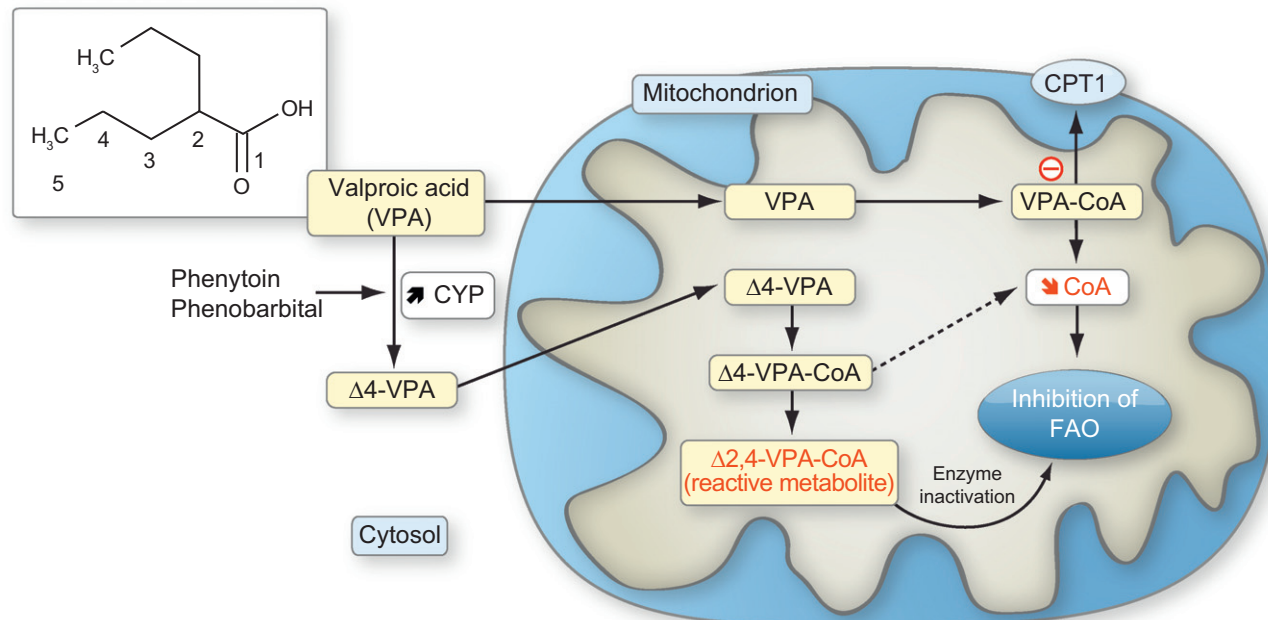


Fig. 3. Mechanisms of valproic acid-induced inhibition of mitochondrial fatty acid β -oxidation. Valproic acid (VPA, or dipropylacetic acid) is an analogue of medium-chain fatty acid which freely enters the mitochondrion and generates a coenzyme A ester (VPA-CoA) within the mitochondrial matrix. This VPA-CoA derivative can inhibit carnitine palmitoyltransferase-1 (CPT 1), an enzyme catalyzing the rate limiting step of the mitochondrial entry and β -oxidation of long-chain fatty acids. Furthermore, the generation of the VPA-CoA ester reduces mitochondrial levels of CoA, which is a cofactor mandatory for fatty acid oxidation (FAO). A second mechanism which could play a major role in VPA-induced inhibition of FAO is the cytochrome P450 (CYP)-mediated generation of $\Delta 4$ -VPA (a VPA metabolite which presents a double bond between carbons 4 and 5, respectively). Indeed this metabolite also enters the mitochondrion to generate $\Delta 2,4$ -VPA-CoA, a reactive metabolite able to covalently bind to (and thus inactivate) FAO enzymes. The generation of $\Delta 4$ -VPA can be enhanced by a co-treatment with phenytoin and phenobarbital which are CYP inducers.

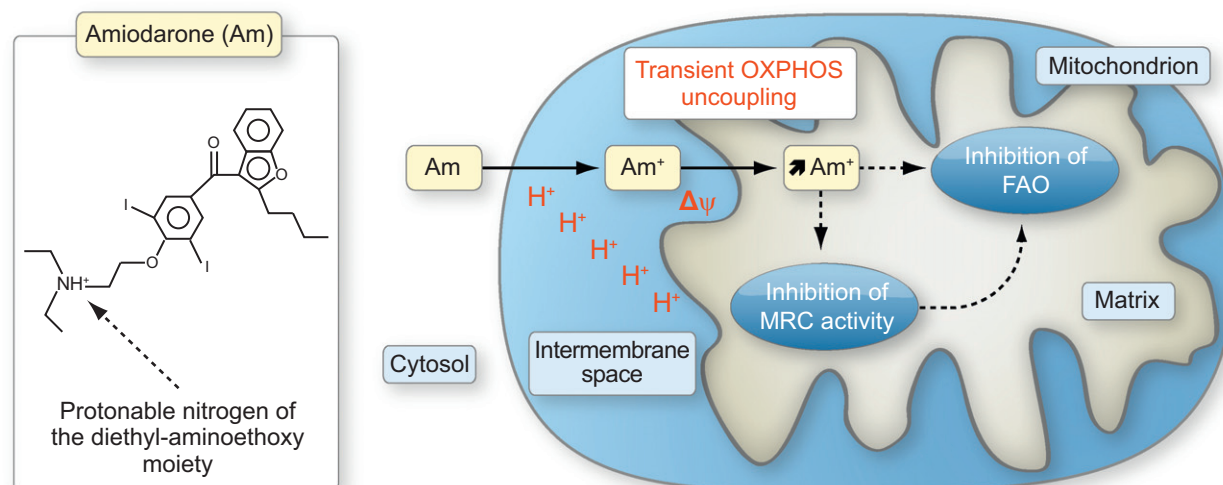


Fig. 4. Mechanisms of amiodarone-induced impairment of oxidative phosphorylation and mitochondrial fatty acid β -oxidation. Amiodarone (Am) is an amphiphilic compound which harbors a protonable nitrogen within its diethyl-aminoethoxy moiety. In the intermembrane space of mitochondria (which is an acidic milieu) Am undergoes a protonation to generate Am^+ . This cationic derivative thus freely enters the mitochondrion thanks to the mitochondrial transmembrane potential $\Delta\psi$. The entry of the protonated molecule Am^+ has two major consequences regarding oxidative phosphorylation (OXPHOS) and mitochondrial fatty acid oxidation (FAO): (1) a rapid and transient uncoupling of OXPHOS since protons are not entering the matrix through ATP synthase; (2) a progressive accumulation of Am^+ within the mitochondrial matrix which induces the subsequent inhibition of different enzymes involved in the mitochondrial respiratory chain (MRC) and FAO. Hence, amiodarone-induced inhibition of FAO could result from the direct inhibition of FAO enzymes (such as CPT 1) and to an impairment of the MRC activity at the level of complexes I and II.

directly FAO enzyme(s), higher concentrations are required in order to impair the MRC [30,99,101,102,106]. Thus, accumulation of these amphiphilic drugs within the mitochondria eventually inhibits FAO through a dual mechanism. Finally, although tetracycline derivatives can also reduce the MRC activity [5,136], it is still unclear whether these drugs inhibit mitochondrial FAO through MRC impairment or by a direct mechanism.

Fourthly, drugs can impair mitochondrial FAO and induce microvesicular steatosis by reducing mtDNA levels (Table 1). Indeed, profound mtDNA depletion induces MRC impairment and secondary inhibition of FAO. This has been shown for the antiviral fialuridine (FIAU), AZT, d4T, and ddI, which all inhibit the mtDNA polymerase γ [5,6,41,137,138]. Low mtDNA levels can also be associated with lactic acidosis resulting from the inhibition of the TCA cycle [6,139,140]. Tamoxifen and tacrine can also induce hepatic mtDNA depletion although it is still unclear whether this mechanism plays a major pathophysiological role [7,96,102]. Both tamoxifen and tacrine reduce mtDNA synthesis by interacting with the mitochondrial topoisomerases [96,102].

Drugs can also induce mtDNA damage through the production of ROS, reactive nitrogen species (RNS) and/or reactive metabolites. For instance APAP and troglitazone can induce mtDNA strand breaks which eventually lead to a reduction of mtDNA levels [141,142]. Indeed, damaged mtDNA molecules harboring numerous strand breaks can be rapidly degraded by mitochondrial endonucleases [143–145]. The antiretroviral NRTIs can also cause the accumulation of the oxidized base 8-hydroxydeoxyguanosine (8-OH-dG) in liver and muscle mtDNA [41,146]. In addition, mtDNA point mutations have been detected in some patients treated with NRTIs. These point mutations may result from the misreading of 8-OH-dG by DNA polymerase γ during mtDNA replication and/or NRTI-induced impairment of polymerase γ repair capacity

[41,147]. Hence, some drugs are liable to cause quantitative and qualitative mtDNA alterations due to their interaction with mitochondrial enzymes involved in mtDNA replication and maintenance and/or through the generation of ROS and reactive metabolites.

Key points 1

Drug-induced mitochondrial dysfunction.

- Mitochondrial dysfunction is a major pathway whereby some drugs and/or their metabolites can induce liver injury.
- Drugs can impair mitochondrial function through several mechanisms including induction of membrane permeabilization, impairment of the oxidative phosphorylation (OXPHOS) process, and inhibition of fatty acid oxidation (FAO).
- Mitochondrial membrane permeabilization and OXPHOS impairment can induce necrosis and/or apoptosis, thus leading to cytolytic hepatitis.
- A severe inhibition of mitochondrial FAO can induce microvesicular steatosis, a liver lesion characterized by an accumulation of small lipid droplets within the hepatocytes and associated with liver failure, encephalopathy, and profound hypoglycemia.
- Impairment of mitochondrial FAO can be direct (e.g. through an inhibition of FAO enzymes) or indirect (e.g. through a reduction of the MRC activity).
- Since 13 proteins of the MRC are encoded by the mitochondrial DNA (mtDNA), drug-induced inhibition of mtDNA replication and/or mtDNA damage can secondarily impair OXPHOS and mitochondrial FAO.

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Table 5. Examples of drugs inducing macrovacuolar steatosis and steatohepatitis.

Drug	Therapeutic class
Amiodarone	Antianginal, antiarrhythmic
5-Fluorouracil	Antineoplastic (colorectal cancer)
Glucocorticoids	Anti-inflammatory
Interferon- α	Antiviral (anti-HCV and anti-HBV)
Irinotecan	Antineoplastic (colorectal cancer)
Methotrexate	Antipsoriatic, anti-rheumatoid
Nifedipine	Antianginal, antihypertensive
NRTIs* (AZT, ddI, d4T)	Antiretroviral (anti-HIV)
Perhexiline	Antianginal
Tamoxifen	Antineoplastic (breast cancer)
Toremifene	Antineoplastic (breast cancer)

*Abbreviation: NRTIs, nucleoside reverse transcriptase inhibitors.

Drug-induced alterations of hepatic lipid metabolism inducing macrovacuolar steatosis

With some drugs (Table 5) [6,148–151], liver triglycerides accumulate as a large (often single) lipid vacuole displacing the

nucleus at the periphery of the hepatocyte. This liver lesion is commonly referred to as macrovacuolar steatosis [6,152]. Several drugs responsible for this hepatic lesion can also induce a mixed form of fat accumulation with macrovacuolar steatosis in some hepatocytes and microvesicular steatosis in others. It is possible that the size of the fat droplets could depend on the nature of some proteins wrapping the lipids (e.g. perilipin and adipophilin) and/or their content in free fatty acids [5,153]. Alternatively, the coexistence of both types of steatosis could result from the occurrence of different mechanisms of toxicity in distinct hepatocytes.

Macrovacuolar steatosis is also observed in a large number of obese and diabetic patients, even in those that do not drink alcohol. That is why it is often referred to as nonalcoholic fatty liver in the context of obesity and related metabolic disorders [60,69,154]. In these disorders, hepatic steatosis primarily results from two mechanisms: 1) an increased delivery of free fatty acids to the liver which is the consequence of insulin resistance in adipose tissue (that favors triglycerides hydrolysis); and, 2) a stimulation of *de novo* hepatic lipogenesis, which is mainly due to hyperinsulinemia and hyperglycemia that activate the transcription factors SREBP-1c and ChREBP, respectively [60,155,156].

Ethanol intoxication frequently induces macrovacuolar steatosis although microvesicular steatosis can be also observed

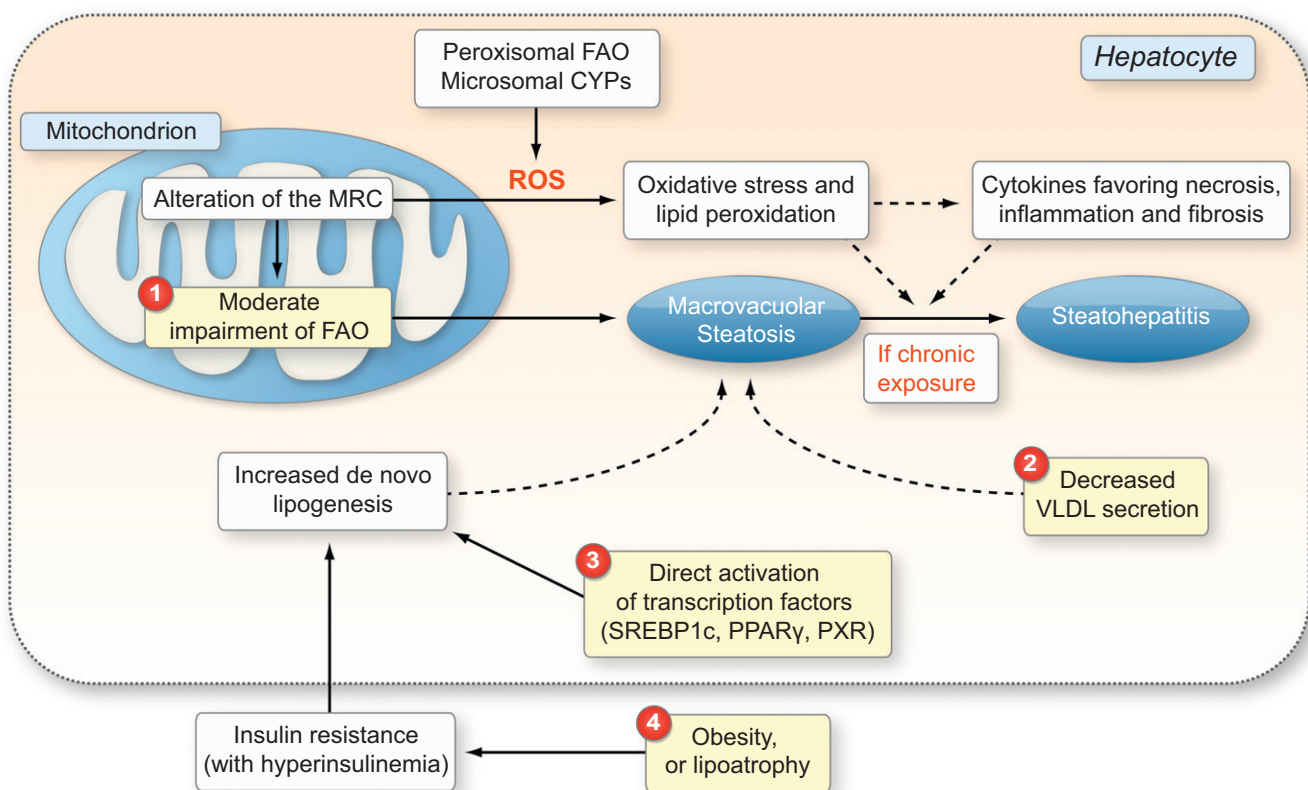


Fig. 5. Mechanisms of drug-induced macrovacuolar steatosis and steatohepatitis. Drugs can induce macrovacuolar steatosis through at least four different mechanisms: (1) by inducing a moderate impairment of mitochondrial fatty acid oxidation (FAO); (2) by decreasing the secretion of very-low density lipoprotein (VLDL); (3) by directly activating transcription factors involved in hepatic lipogenesis, such as SREBP-1c, PPAR γ , and PXR, and; (4) by favoring the occurrence of insulin resistance and hyperinsulinemia, which can be the consequence of obesity or lipoatrophy (i.e. a reduction of body fatness). It is noteworthy that the progression of steatosis into steatohepatitis in some patients involves the production of reactive oxygen species (ROS), which is responsible for oxidative stress and lipid peroxidation. These deleterious events subsequently trigger the production of different cytokines such as TNF α and TGF β that favor necroinflammation and fibrosis. Although the mitochondria produce the majority of ROS through the alteration of the mitochondrial respiratory chain (MRC), other sources could involve peroxisomal FAO and microsomal cytochromes P450 (CYPs).

[5,157]. Ethanol-induced fatty liver results from different mechanisms including increased hepatic uptake of fatty acids and *de novo* lipogenesis, impaired PPAR α signaling, mitochondrial dysfunction and reduced secretion of triglycerides [5,158–161]. Some of these effects could be due to reduced adiponectin secretion by the adipose tissue and elevated expression of tumor necrosis factor- α (TNF α), which both favor lipid synthesis and reduced mitochondrial FAO [162–164].

Regarding drug-induced macrovacuolar steatosis, different mechanisms seem involved (Fig. 5), and a single molecule can alter several metabolic pathways.

Firstly, a moderate inhibition of mitochondrial FAO could play a role with amiodarone, perhexiline, tamoxifen, NRTIs, and glucocorticoids [5,6,102,165,166]. However, some of these drugs could induce stronger inhibition of mitochondrial FAO in a few patients thus leading to the occurrence of microvesicular steatosis, as previously mentioned.

Secondly, a reduction of hepatic VLDL secretion has been described with amiodarone, perhexiline, and tetracycline which all inhibit MTP activity [5,124]. D4T was shown to reduce MTP mRNA expression in cultured rat hepatocytes but MTP activity was not assessed [167]. Interestingly, small molecules inhibiting MTP have been tested in order to lower blood lipids, but the clinical usefulness of this therapeutic strategy has been hampered by their potential to induce hepatic steatosis [168,169].

Thirdly, increased cellular uptake of fatty acids could play a significant role with some compounds. This mechanism has been proposed for efavirenz which activates AMP-activated protein kinase (AMPK) most probably as a consequence of mitochondrial complex I inhibition and reduced ATP synthesis [170]. Indeed, AMPK activation promotes fatty acid uptake into the cell through the fatty acid transporter FAT/CD36 in addition to its stimulating role on mitochondrial FAO [171]. Thus, efavirenz-induced lipid accumulation in hepatocytes is likely favored by the concomitant increased uptake of extracellular fatty acids and impaired mitochondrial FAO [170].

Fourthly, a stimulation of hepatic lipid synthesis could be involved with drugs, such as interferon- α , glucocorticoids, tamoxifen, troglitazone, and nifedipine [172–175]. Although the mechanisms whereby these drugs favor lipid synthesis are not precisely known, some of them could activate lipogenic transcription factors thus leading to the subsequent induction of enzymes, such as ACC and fatty acid synthase [165,175,176]. At least three transcription factors could be involved in drug-induced activation of lipogenesis: (1) PXR, which could play a role with nifedipine, tamoxifen, and troglitazone as these drugs are PXR activators [62,177,178]; (2) PPAR γ , which could be involved with the PPAR γ ligand troglitazone [176]. Actually, thiazolidinediones (TZDs) could favor lipid accretion and worsen liver function more easily in the context of pre-existent induction of PPAR γ expression [179,180], as discussed in the next section; (3) Glucocorticoid receptor (GR) whose activation plays a central role in glucocorticoid-induced hepatic lipogenesis and steatosis [165,181]. Finally, some investigations suggest that the activation of the constitutive androstane receptor (CAR) could play a role in phenobarbital-induced hepatic steatosis [182]. However, steatosis is rarely observed in patients treated with phenobarbital [1], and liver fat accumulation in mice is only transient and disappears after 1 week of treatment with this CAR activator [182]. In addition, CAR activation with 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) reduces hepatic lipogenesis and

prevents fatty liver induced by obesity or a methionine choline-deficient diet [183–185]. Hence, the nuclear receptor CAR may have divergent effects on hepatic lipogenesis depending of the duration of its activation and/or the nature of its activator.

Key points 2

Drug-induced lipid dysmetabolism and macrovacuolar steatosis.

- Besides inducing mitochondrial dysfunction, drugs can also alter lipid metabolism in liver by increasing *de novo* fatty acid synthesis and decreasing the secretion of VLDL-associated triglycerides.
- In this context, lipids accumulate within the hepatocytes as large vacuoles thus leading to macrovacuolar steatosis (also referred to as fatty liver).
- Some drugs can favor macrovacuolar steatosis by inducing either obesity and insulin resistance, or lipoatrophy (i.e. a reduction of body fat mass).
- Drug-induced augmentation of hepatic lipid synthesis can be due to a direct activation of lipogenic transcription factors, or to hormonal dysregulation (e.g. hyperinsulinemia and hypoleptinemia).
- Several drugs inducing microvesicular steatosis in a few patients can also favor the occurrence of macrovacuolar steatosis in others depending on the severity of mitochondrial FAO inhibition and/or the induction of other disturbances of lipid homeostasis.

Mechanisms involved in the progression of steatosis into steatohepatitis

Several drugs can induce steatohepatitis (Table 5) [5,6,116,149,186–189], a potentially severe liver lesion characterized by the presence of necroinflammation, fibrosis, and Mallory bodies. In the context of drug-induced steatohepatitis, fat accumulates usually as large vacuoles, although microvesicular steatosis can also be present in some hepatocytes. Inflammation and fibrosis can be of variable severity and occasionally cirrhosis occurs with drugs, such as amiodarone, perhexiline, and didanosine [116,149,190–192]. Importantly, drug-induced steatohepatitis shares many pathological and clinical features with alcoholic steatohepatitis and nonalcoholic steatohepatitis (NASH).

Although there are still some unsolved issues about the mechanisms involved in the progression of steatosis into steatohepatitis, there is evidence for a key role of mitochondrial dysfunction (Fig. 5). Indeed, several drugs causing steatohepatitis are able to impair the mitochondrial OXPHOS process and inhibit the MRC (Fig. 5) [5–7,193]. Actually, inhibition of the MRC could not only participate to fat deposition but also to ROS overproduction. However, other (i.e. nonmitochondrial) sources of ROS are probably involved, such as peroxisomal FAO, or microsomal CYPs [194,195].

ROS, whatever their sources, can then trigger peroxidation of polyunsaturated fatty acids, a degradative process generating reactive aldehydic derivatives, such as malondialdehyde and 4-hydroxynonenal [195–197]. Importantly, ROS and lipid

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peroxidation products activate Kupffer and stellate cells that play a role in inflammation and fibrogenesis, respectively (Fig. 5) [155,196,198–201]. Lipid peroxidation products are also able to modulate stress signaling pathways, damage DNA (including mtDNA), inhibit MRC activity and induce cell death [202–206]. Interestingly, malondialdehyde can cross-link cytokeratine 8, which may contribute to Mallory bodies' formation [207]. ROS and lipid peroxidation-induced MRC impairment and mtDNA damage also promote mitochondrial dysfunction, thus leading to a vicious circle, which can further increase ROS production and provoke cell death. Finally, the production by activated inflammatory cells of several cytokines, such as TNF α and TGF β can also participate in cell death during steatohepatitis (Fig. 5) [7,155,208].

Some drugs, such as tamoxifen, irinotecan, methotrexate, and the TZDs pioglitazone and rosiglitazone could aggravate the pre-existing nonalcoholic fatty liver disease (NAFLD) in obese and diabetic patients, and sometimes hasten the progression of steatosis into steatohepatitis and severe fibrosis [180,209,210–212]. Although the mechanisms involved in drug-induced aggravation of pre-existing NAFLD in obese patients are not known, some hypotheses can be put forward. For instance, activation of PPAR γ and *de novo* lipogenesis could be involved with the TZDs [176,180]. Indeed, although PPAR γ expression is low (or nil) in normal liver it could be enhanced in liver presenting NAFLD [69,213–215], thus allowing its full-blown activation by the synthetic PPAR γ ligands. Alternatively, some of these drugs could worsen the pre-existing mitochondrial dysfunction present in NAFLD [155,216]. This may occur with tamoxifen and methotrexate which both impair MRC activity [102,193,217]. Finally, cigarette smoke exposure and chronic ethanol intoxication could also aggravate NAFLD in the context of obesity [218–220].

Key points 3

Steatohepatitis and factors favoring drug-induced mitochondrial and metabolic toxicity.

- While macrovacuolar steatosis is a relatively benign liver lesion in the short term, it can progress into steatohepatitis after several months (or years) of treatment.
- Whatever the etiology of liver steatosis, its progression into steatohepatitis involves mitochondrial dysfunction, lipid peroxidation, and overproduction of reactive oxygen species.
- Some drugs inducing steatohepatitis can also cause cirrhosis in a few patients.
- Numerous factors are able to favor drug-induced mitochondrial and metabolic toxicity, such as the structure of the parent molecules, genetic predispositions, alcoholic intoxication, hepatitis virus C infection, and obesity.
- In the context of obesity and related metabolic disorders, some drugs could induce acute liver injury more frequently while others could worsen the pre-existent steatohepatitis.

Drug-induced lysosomal phospholipidosis

Drugs such as amiodarone and perhexiline can induce liver phospholipidosis, which is characterized by an accumulation of phospholipids within the lysosomes, thus leading to the formation of "lamellar bodies" in affected hepatocytes [221,222]. Drug-induced phospholipidosis is frequent and has apparently few

(or no) biochemical or clinical consequences if it is not associated with other histopathological alterations [5,223]. At least two mechanisms could be involved in drug-induced phospholipidosis including a decline of intracellular lysosomal enzyme levels and an inhibition of several lysosomal phospholipases [5,221,224]. Interestingly, investigations showed that amiodarone and perhexiline-induced effects on mitochondria and lysosomes are related to their chemical structure. Indeed, these amphiphilic drugs can be protonated in the intermembrane space of mitochondria or inside the lysosomes that are both acidic milieus. This protonation generates cationic molecules that accumulate within the mitochondria and inhibit MRC and FAO enzymes (as previously discussed), or interact with intralysosomal phospholipids, thus inhibiting the action of phospholipases [5,30,221].

Drug-induced hepatic steatosis through adipose tissue alterations and insulin resistance

Some drugs could favor fatty liver by altering the white adipose tissue (WAT) (Table 6). This situation occurs for instance with d4T and ddI which can induce lipoatrophy (i.e. reduction of body fat mass) and a subsequent reduction of leptin secretion by the white adipocytes [225,226]. Indeed, low leptinemia enhances *de novo* lipogenesis in the liver, as already mentioned [69,227]. In addition, hypoleptinemia likely promotes lipid accretion in skeletal muscle and pancreas, thus causing insulin resistance and type 2 diabetes (Fig. 5) [228,229]. Consequently, both hypoleptinemia and subsequent insulin resistance could favor liver lipid accumulation in patients suffering from NRTI-induced lipoatrophy [225–227].

In contrast, some drugs promote steatosis and steatohepatitis by increasing body fatness (Table 6). In this context, insulin resistance and subsequent hyperinsulinemia induce hepatic lipid accumulation [60,156]. This scenario occurs with glucocorticoids, which cause central obesity, at least in part as a result of CNS-mediated increase in food intake [230]. Glucocorticoid-induced obesity can be associated with insulin resistance, diabetes, dyslipidemia, and fatty liver, as previously mentioned [174,231,232]. Glucocorticoids could also promote hypoadiponectinemia and related metabolic disturbance through a mechanism unrelated to the expansion of body fat mass [233]. Tacrolimus (another immunosuppressive drug) favors hepatic steatosis in some liver transplant recipients through reduced pancreatic insulin secretion and secondary diabetes [234,235].

The antipsychotic drugs clozapine, olanzapine, chlorpromazine, and risperidone can increase food intake and induce obesity through mechanisms that may involve interaction with the serotonergic 5-HT $_2C$ receptors and/or disruption of leptin signaling in the hypothalamus [236,237]. Besides increasing appetite through CNS actions, some of these drugs could also directly favor lipogenesis in adipocytes [238–240]. Importantly, antipsychotics-induced obesity can be associated with various metabolic disorders, such as insulin resistance, diabetes, dyslipidemia, and fatty liver [237,241–244]. Although antipsychotics-induced fatty liver could be an indirect consequence of obesity and insulin resistance, experimental studies showed that drugs such as clozapine and olanzapine directly increase *de novo* lipogenesis in hepatocytes [245]. SREBP activation could be a common mechanism whereby some antipsychotic drugs directly trigger lipogenesis in both adipocytes and hepatocytes [240,245,246].

Table 6. Examples of drugs inducing obesity, or lipoatrophy, thus favoring the occurrence of insulin resistance and NAFLD.

Drug	Obesity/Lipoatrophy	Therapeutic class
Glucocorticoids	Obesity	Anti-inflammatory
Olanzapine	Obesity	Antipsychotic
NRTIs ^a (ddI, d4T)	Lipoatrophy	Antiretroviral (anti-HIV)
Protease inhibitors	Lipoatrophy	Antiretroviral (anti-HIV)
Risperidone	Obesity	Antipsychotic
Valproic acid (VPA)	Obesity	Antiepileptic drug

^aAbbreviation: NRTIs, nucleoside reverse transcriptase inhibitors.

Occurrence of obesity is also a great concern in patients treated with VPA [247,248], which could stimulate appetite directly through a hypothalamic effect and indirectly by impairing leptin secretion or bioavailability [247,249,250]. Actually, macrovesicular steatosis seems highly prevalent in VPA-treated patients and liver fat accretion is positively correlated with body mass index and plasma insulin levels [251,252]. In addition, steatohepatitis can also occur in patients treated with VPA [253,254]. Hence, the high prevalence of hepatic steatosis in VPA-treated patients is likely related to its propensity to induce obesity and insulin resistance. However, one cannot exclude a direct detrimental effect of this drug on hepatic mitochondrial FAO, as previously discussed.

Finally, it is noteworthy that ethanol intoxication could favor fatty liver and steatohepatitis through reduced adiponectin secretion [162,255]. As adiponectin presents anti-steatotic and anti-inflammatory action, reduced plasma adiponectin in alcoholics could favor both hepatic lipid accretion and necroinflammation. Liver dysfunction resulting from hypoadiponectinemia adds to the numerous deleterious effects directly induced by ethanol intoxication in hepatocytes including oxidative stress, lipid peroxidation, and mitochondrial dysfunction [5,7,256]. However, moderate ethanol consumption enhances plasma adiponectin levels and this may explain, at least in part, why reasonable alcohol intake affords favorable effects on obesity-associated fatty liver and type 2 diabetes [257–259].

Factors favoring drug-induced toxicity on mitochondria and lipid metabolism

Numerous factors may favor drug-induced mitochondrial and metabolic toxicity in treated patients and only the most important of them will be mentioned below.

Drug structure and metabolism

Chemical structure and intrahepatic metabolism play a major role for several drugs. Amiodarone, perhexiline, tamoxifen, and buprenorphine are amphiphilic drugs harboring protonable amine moieties that favor their accumulation inside the mitochondrial matrix under the influence of the membrane potential $\Delta\psi_m$ (Fig. 4) [7,30,99,101,102]. VPA (dipropylacetic acid) is a branched-chain fatty acid activated by coenzyme A, thus explaining why this drug can reduce the intracellular levels of this mitochondrial FAO cofactor (Fig. 3) [5,7,135]. In addition, CYP-mediated biotransformation of VPA into $\Delta 4$ -VPA subse-

quently gives rise to $\Delta 2,4$ -VPA-CoA and other reactive metabolites that irreversibly inactivate FAO enzymes (Fig. 3) [5,129,130]. This contribution of CYPs in VPA-induced mitochondrial toxicity explains in large part why its hepatotoxicity is favored by the concomitant administration of CYP inducers such as phenobarbital and phenytoin (Fig. 3) [5,7,260]. Finally, NRTIs inhibit mtDNA replication due to their ability to undergo phosphorylation as the cognate endogenous nucleosides and to be subsequently incorporated within the mitochondrial genome by the DNA polymerase γ [41,147].

Drug dosage and duration of the treatment

Clinical reports in the 50's and 60's indicated that severe microvesicular steatosis induced by tetracycline and its derivatives was clearly dose-dependent [5]. In particular, most cases of fatty liver were observed in patients receiving large intravenous dosages (>1.5 g/day) of tetracycline derivatives [5,261]. However, tetracycline-induced steatosis is no longer observed since such huge intravenous doses have been abandoned. Regarding VPA, asymptomatic elevation of transaminases can be normalized by reducing its dosage but VPA-induced microvesicular steatosis does not appear to be dose-dependent [5,262]. Long-term administration of amiodarone and perhexiline could also favor steatohepatitis, a liver lesion which usually occurs after several months or years of treatment [5,149]. Amiodarone accumulates in numerous tissues of treated patients including liver, lung, and adipose tissue and can be detectable in plasma several months after its discontinuation [189,263,264]. Hence, amiodarone-induced hepatotoxicity can further deteriorate despite stopping the antiarrhythmic therapy [265]. Long-lasting administration of NRTIs also increases the risk of mitochondrial toxicity in liver and adipose tissue [266,267].

Genetic predispositions

Several genetic predispositions could enhance the risk of drug-induced mitochondrial toxicity and subsequent liver injury. Conceptually, DNA polymorphisms (or sometimes mutations) can favor DILI through different mechanisms including: (1) accumulation of the potentially toxic parent drug via reduced activity of drug-metabolizing enzymes such as CYPs; (2) increased levels of reactive metabolite(s) and oxidative stress due to lower activity of enzymes involved in drug or ROS detoxication; and (3) mild pre-existent mitochondrial dysfunction that can be deteriorated during the course of the treatment. Several examples of gene alteration predisposing for mitochondrial/metabolic toxicity and DILI are given below.

In patients treated with perhexiline, polymorphism in the *CYP2D6* gene may favor steatohepatitis and cirrhosis through a reduction of its oxidation [5,268]. A polymorphism in the *CYP17* gene, which regulates serum estrogen, has been associated with an increased risk of tamoxifen-induced hepatic steatosis [269]. The risk of troglitazone-induced hepatotoxicity was enhanced in patients harboring the combined glutathione S-transferase *GSTT1-GSTM1* null genotype [270], whereas the same genotype was found to increase the susceptibility of liver injury induced by other drugs [271]. As these GSTs seem to be involved in the detoxication of an epoxide metabolite of troglitazone, their deficiency may promote the accumulation of this reactive intermediate and subsequent liver toxicity [270]. On the contrary, *CYP2C9*

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genetic polymorphism may reduce the formation of $\Delta 4$ -VPA and thus the likelihood of VPA-induced hepatotoxicity [272].

Several congenital defects in mitochondrial enzymes involved in FAO and OXPHOS have been detected in patients with VPA hepatotoxicity [5,273–275]. This drug also induced more frequently liver injury in patients harboring mutations (e.g. A467T, W748S and Q1236H) in the gene encoding DNA polymerase γ [276,277]. Another mutation (R964C) in the gene encoding DNA polymerase γ may also favor mitochondrial toxicity induced by NRTIs, possibly by enhancing the probability of their incorporation within the mtDNA molecules and the subsequent arrest of mtDNA replication [278,279]. Inter-individual differences in mitochondrial anti-oxidant enzymes such as MnSOD may increase the risk of mitochondrial oxidative damage and hepatotoxicity induced by different drugs and alcoholic intoxication [280–283]. Finally, some genetic factors may augment the risk of drug-induced obesity, insulin resistance, and dyslipidemia [237,284–286], thus indirectly promoting the occurrence of fatty liver.

Obesity and type 2 diabetes

There is growing evidence that obesity can increase the risk of DILI, at least for some drugs. In fact, two distinct clinical settings may exist. Firstly, obese patients could be more prone to develop drug-induced acute hepatitis. This has been suggested for the volatile halogenated anaesthetic halothane [287–289], APAP [290,291], and different drugs, such as losartan, ticlopidine, and omeprazole [292]. Interestingly, it has been reported that diabetes also increases the risk of acute liver failure (ALF), including drug-induced ALF [293]. Secondly, the pre-existing NAFLD observed in obese and diabetic individuals could be further aggravated by the chronic intake of drugs, such as tamoxifen [209], irinotecan [151,210], NRTIs, [267] and methotrexate [294,295]. However, obesity may not increase the risk of DILI for all potential hepatotoxic drugs. For instance, amiodarone may not be more hepatotoxic in obese patients with a metabolic syndrome [296].

Experimental studies have dealt with the issue of xenobiotic-induced hepatotoxicity in the context of obesity. Unfortunately, the mechanisms of enhanced liver sensitivity have not always been determined. For instance, hepatotoxicity has been found more severe in obese rodents treated with tetracycline [297], phenobarbital, [298] and haloperidol [299], but no mechanistic explanations were provided in these studies. As previously mentioned, activation of PPAR γ could explain why the TZD rosiglitazone aggravated NASH in obese ob/ob mice [180]. Studies in rodents have shown that obesity also favors hepatotoxicity induced by binge ethanol exposures through mechanisms involving increased expression of TNF α and Fas ligand [220,300]. Hence, NAFLD could be aggravated by drugs through different mechanisms including an enhanced ability of the obese liver to synthesize fat and to produce cytokines promoting necroinflammation and fibrosis. Other common mechanisms may be based on reduced anti-oxidant defenses with lower GSH levels and GST expression [301,302], as well as latent MRC dysfunction [60,155,216].

For halothane and APAP, a specific mechanism could be an increased activity of hepatic CYP2E1, which is the main CYP isoenzyme involved in the generation of their toxic reactive metabolites [303–306]. Indeed, CYP2E1 expression and activity are

enhanced in obese patients, in particular in those with NAFLD, although the exact mechanism of CYP2E1 induction is still poorly understood [307–310]. When compared to lean individuals morbidly obese patients tended to have higher plasma levels of trifluoroacetic acid, the end product of CYP2E1-mediated oxidation of halothane, which reflects the generation of the reactive metabolite trichloroacetyl chloride [311]. Unfortunately, hepatic CYP2E1 activity was not assessed in this study. As regards APAP, although different investigations dealt with the effect of obesity on its disposition it is still unknown whether the toxic metabolite NAPQI is generated at a greater extent in obese patients [312–314]. Finally, investigations in obese animals treated with APAP have given conflicting results with either increased hepatotoxicity [315,316] or an obvious protection [317,318]. Although the reasons of these discrepancies are still unclear, protection against APAP-induced liver toxicity was observed in obese ob/ob mice and fa/fa Zucker rats that consistently present normal, or even reduced, hepatic CYP2E1 expression and activity [220,300,319–322].

Hepatitis C virus infection and alcohol intoxication

Other factors such as hepatitis C virus (HCV) and alcoholic intoxication can enhance the risk of DILI, in particular during NRTI therapy [323,324]. Interestingly, both factors induce mitochondrial dysfunction and oxidative stress [5,256,325–327]. These factors also disturb lipid metabolism beyond their deleterious effects on mitochondrial function. Whereas HCV impairs hepatic VLDL secretion and induces insulin resistance [328,329], alcoholic intoxication strongly enhances hepatic lipogenesis through SREBP-1c activation [160,161,164,327].

Alcoholic intoxication could also favor hepatotoxicity with methotrexate, buprenorphine and APAP [7,330,331]. Although chronic heavy alcohol consumption enhances the risk of APAP-induced liver injury in the context of APAP overdose, some cases of hepatotoxicity have also been reported in alcoholics taking modest doses of APAP [330,332]. Ethanol overconsumption could favor APAP-induced liver injury through at least three different mechanisms: (1) CYP2E1 induction; (2) reduction of GSH stores; and (3) damage of mitochondrial components including MRC complexes and mtDNA [6,333,334]. CYP2E1 induction enhances the biotransformation of APAP into NAPQI, a particularly reactive metabolite that binds covalently to endogenous molecules, such as DNA, some polypeptides (in particular within the mitochondria), and GSH. The covalent binding of large amounts of NAPQI to GSH thus induces a massive reduction of its intracellular levels and subsequent oxidative stress, which can reinforce mitochondrial dysfunction [34,90,92,303]. Hence, APAP-induced oxidative stress and cell demise are favored when GSH stores are reduced by previous alcohol intoxication. Finally, it is noteworthy that a significant amount of hepatic CYP2E1 is located within the mitochondria, in particular after ethanol intake [54–56,321]. Thus, NAPQI could be directly generated within liver mitochondria in the context of prior alcoholic overconsumption.

Remaining issues and concluding remarks

Numerous drugs can be toxic for the liver [1] and hepatic mitochondria seem to be preferential targets (Table 1). However, more investigations are needed to determine the precise list of drugs inducing mitochondrial dysfunction and subsequent liver lesions.

To address this major issue it is urgent to set up high-throughput technologies [335], which could help to rapidly screen a great number of molecules. This screening is also important for the early detection of mitochondrial toxicity during preclinical studies since it can avoid late-stage withdrawal during drug development [6,78,336].

More than a decade ago, drug-induced steatosis was mainly considered as the consequence of impaired mitochondrial FAO [5,337]. Although this concept remains valid for microvesicular steatosis, recent investigations clearly indicate that drug-induced macrovesicular steatosis can be due to several mechanisms including reduced VLDL export, enhancement of *de novo* lipogenesis and alteration of body fatness. The latter mechanism illustrates the concept that some drugs can indirectly damage the liver by increasing (or less frequently, decreasing) body fat mass, thus inducing insulin resistance and altering the secretion of adiponectin and leptin. This is a challenging issue since such indirect mechanisms of liver injury cannot be detected thanks to *in vitro* investigations. Because fatty liver can progress into steatohepatitis and cirrhosis, this lesion cannot be deemed as benign in the long-term. Moreover, recent investigations also suggest that obese individuals could present a greater risk of DILI although this could involve some (but not all) drugs. Thus, it has become clear that the adipose tissue plays a role in DILI. As there are millions of obese individuals taking drugs on a regular basis more investigations are needed to determine the exact impact of obesity on drug safety, in particular regarding the liver.

Circadian rhythms significantly change gene expression in different tissues including the liver [338,339]. Recent experimental investigations suggest that these circadian rhythms may modulate the incidence and severity of drug-induced hepatotoxicity, in particular by modifying CYP expression [340,341]. However, clinical investigations will be required to translate these results to the human situation. Disruption in circadian rhythmicity may also have various detrimental effects regarding carbohydrate and lipid homeostasis in the liver [342,343]. Since some drugs can alter the hepatic expression of clock genes [344,345], it will be interesting to determine whether these changes favor the occurrence of steatosis and steatohepatitis.

Another major issue is the identification of the main factors increasing the risk of DILI. Since numerous cases of DILI may be idiosyncratic (i.e. host-dependent), it will be important to identify these factors in order to reduce the frequency of side effects [346,347]. Although some congenital and acquired factors that modify mitochondrial/metabolic homeostasis have already been detected, there are many others that need to be uncovered. While large-scale prospective human studies will be required to solve this issue, investigations in appropriate animal models will also be useful [78,348–350].

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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